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The Effect of Ethanol Extract of Phaleria macrocarpa Fruit Combined with Deferiprone on Peripheral Blood Counts in Iron-Overloaded Rats

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A B S T R A C T

Iron overload poses health risks due to its role in reactive oxygen species (ROS) formation.This condition is commonly found in patients with thalassemia due to ineffective erythropoiesis and repeated blood transfusions. Previous studies have shown that excess iron can cause damage, including to the hematological system. Meanwhile, iron chelation therapy with deferiprone, a standard chelator for managing iron overload, is also known to have hematological side effects. We evaluated the efficacy of ethanol extract of *Phaleria macrocarpa* Fruit (PM) against deferiprone-induced alterations in hematological parameters in iron-overloaded rats. Six groups were studied: control, iron-overloaded (IO), deferiprone (D), PM, and two combination groups (DPM-1 and DPM-2). Hematological parameters were assessed at baseline (week-3) and post-treatment (week-8), including total white blood cell count (WBC), lymphocytes (LYM), granulocytes (GRAN), platelet count (PLT), red blood cell count (RBC), hemoglobin (Hb), mean corpuscular volume (MCV, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Iron overload induced mild changes, with significant increases in MCV, alongside non-significant increasing trend in LYM and decreasing trends in other parameters. Deferiprone led to significant decreases in RBC and Hb, with non-significant increases in MCV and non-significant decreases in other parameters. PM group showed significant decreased in PLT, RBC, and Hb, and a significant increase in MCV and MCH, accompanied by non-significant increase in MCHC and non-significant decreasing trends in WBC, LYM, and GRAN. Combination treatment of ethanol extract of *Phaleria macrocarpa* fruit with deferiprone at usual dose (DPM-1) resulted in significant changes, including decreases in GRAN, RBC, Hb and MCHC and increasing MCV, accompanied by non-significant increase in MCH and non-significant decrease in other parameters. The parameter changes are less pronounced in the DPM-2 group, where the dose of deferiprone is lower, compared to the DPM-1 group. PM alone exhibited minimal effects on hematological parameters compared to deferiprone (except for PLT), indicating the need for further research to elucidate the specific cellular and molecular pathways influenced by these treatments to support the use of PM as adjunct therapy in patients with iron overload.

Keywords: deferiprone, hematology, iron chelator, iron overload, *Phaleria macrocarpa*

INTRODUCTION

Iron, a transition metal, serves as a crucial micronutrient for humans. Similar to many other metals, iron exhibits various oxidation states, with ferrous (Fe2+) and ferric (Fe3+) being the most common. Within the human body, iron plays a vital role in regulating essential biological processes, including various redox reactions, cell proliferation, and DNA synthesis (1). Additionally, iron functions as an integral component of hemoglobin and myoglobin, facilitating oxygen binding and transportation (2). Despite its importance, excess iron (iron overload) can lead to the formation of reactive oxygen species (ROS), resulting in damage to DNA, proteins, and lipids (3).

Thalassemia stands as the most prevalent cause of secondary iron overload (4). In Indonesia, approximately 3-10% of the total population carries the β-thalassemia gene, and 2.6-11.0% carry the α-thalassemia gene. With a birth rate of around 20% annually and a population of 200 million, an estimated 2,500 new cases of β-thalassemia major are predicted each year (5). In thalassemia cases, mutations result in decreased synthesis of α/β hemoglobin chains, rendering erythropoiesis ineffective. This leads to increased iron absorption in the intestines as a response to enhanced erythropoiesis. Iron excess in thalassemia patients is also exacerbated by regular blood transfusions performed to correct anemia caused by ineffective erythropoiesis (6).

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Several studies indicate that iron overload exerts suppressive effects on hematopoiesis. The impairment of hematopoiesis is attributed to the damage inflicted on hematopoietic cells and the hematopoietic microenvironment. Chai et al.'s research on mice revealed that increased reactive oxygen species (ROS) resulting from iron overload reduced the clonogenic function of hematopoietic stem and progenitor cells (HSPCs), as evidenced by a decrease in hematopoietic colony-forming counts (7). Zhou et al.'s study demonstrated that induced iron overload in mice through four weeks of iron-dextran administration led to a decrease in hemoglobin (Hb), platelet count, and white blood cell (WBC) count. Iron overload in this study resulted in reduced viability and proliferation activity of bone marrow mononuclear cells (8).

In efforts to mitigate iron overload, thalassemia patients receive iron chelators (9). Presently, commonly used iron chelators encompass deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX). In Indonesia, the accessibility and affordability of these medications pose challenges, with a majority of patients (63.7%) being administered DFP (10,11). However, the principal drawback associated with the clinical application of DFP is its hematologic adverse events, including neutropenia, agranulocytosis, and thrombocytopenia (12).

Research conducted by Kittipoom et al. revealed that 2.8% of thalassemia patients treated with deferiprone experienced agranulocytosis (13). Another study by Panigrahi et al. identified cytopenia as the second most frequent adverse effect, occurring in 7.2% of patients. Within this study, 4.5% experienced neutropenia/agranulocytosis leading to therapy discontinuation, while three patients encountered transient mild leucopenia or thrombocytopenia, allowing for the continued use of DFP treatment (14). Hu et al.'s research indicated that deferiprone exhibits toxicity towards hematopoietic stem and progenitor cells (HPSC) (15). Although relatively rare, hematological abnormalities resulting from deferiprone administration, particularly agranulocytosis, can have fatal consequences, as reported by Mainou et al.(16). The adverse effects on the hematological system necessitate regular monitoring of the complete blood count, with a particular focus on neutrophil levels. Tricta et al. emphasized the importance of weekly monitoring of the absolute neutrophil count (ANC) for all deferiprone users to reduce the risk of developing agranulocytosis and its potential complications. Additionally, discontinuation of therapy is recommended at the first sign of infection or neutropenia (ANC < 1.5 × 109/L), and rechallenge is to be avoided. However, it's acknowledged that this monitoring protocol may pose a burden for some patients (17).

Given the identified challenges with current iron chelators, particularly their hematological side effects, exploring alternative drugs, especially those derived from domestic sources, becomes imperative. *Phaleria macrocarpa* emerges as a promising natural resource proven to reduce iron levels in both serum and organs. The extract from *Phaleria macrocarpa* fruit contains various beneficial compounds, among which mangiferin has demonstrated its ability to decrease iron levels in the plasma of iron-overloaded rats by up to 60% and enhance its excretion in urine (18). Research by Muruganandan and Gupta highlighted the protective effects of mangiferin against cyclophosphamide-induced erythrocytopenia and leucopenia in rats. In vitro studies demonstrated that mangiferin enhanced the survival of lymphocytes exposed to H2O2 (19). Previous research findings indicate that besides mangiferin, extracts of *Phaleria macrocarpa* fruit also contain various other compounds such as quercetin, naringin (20), naringenin, and epigallocatechin-3-gallate (21), all of which have been demonstrated to possess antioxidant effects and are capable of reducing oxidative stress in various cells or organs (22-27).

Verna et al. compared the effects of deferiprone, mangiferin, and ethanol extract of *Phaleria macrocarpa* fruit extract administered individually to rats with iron overload condition. Their study demonstrated that the administration of 100mg/kgBW ethanol extract of *Phaleria macrocarpa* fruit significantly lowered plasma iron levels(28). Hypothesizing that the administration of ethanol extract of *Phaleria macrocarpa* fruit at 100 mg/kgBW can improve hematologic profiles in iron-overloaded rats by reducing body iron levels and counteracting free radicals through its antioxidant capabilities, we also anticipate that combining ethanol extract of *Phaleria macrocarpa* fruit with deferiprone may mitigate the hematological side effects induced by deferiprone. To investigate this hypothesis, we generated iron-overloaded rats and analyzed peripheral blood parameters in these rats.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this research had been obtained from the Ethics Committee of the Faculty of Medicine, University of Indonesia-Cipto Mangunkusumo Hospital (No.KET-964/UN2.F1/ETIK/PPM.00.02/2022)

Experimental Animals and Sample Size

Animals used in this study were healthy, male Sprague-Dawley rats of ± 8 weeks old with an average body weight of 200-250 grams. The animals were obtained from the Indonesian Food and Drug Authority (BPOM) Jakarta. Maintenance and treatment of experimental animals was carried out at Animal Research facilities, Indonesia Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia. Hematological analysis was carried out in Pharmacokinetic Laboratory, Faculty of Medicine, Universitas Indonesia.

The sample size was determined using Federer's equation for an experiment with six treatment groups (refer to the Study Design and Groups section). According to the calculation, a minimum of four rats per group was required. To account for potential rat mortality during the study, each group's sample size was increased to five rats, resulting in a total of 30 rats.

Study Design and Groups

The research conducted was an in-vivo experimental research with a parallel design. Animals were randomly divided into 6 treatment groups:

1. Normal group (N): normal rats and with no intervention.
2. Iron overload (IO) group: iron-overloaded rats treated with aquadest (negative control group).
3. Defereriprone group (D): iron-overloaded rats treated with deferiprone equivalent to the usual dose in humans (462.5 mg/kg body weight of mice; positive control group).
4. *Phaleria macrocarpa* extract (PM) group: iron-overloaded rats treated with ethanol extract of *Phaleria macrocarpa* fruit (100 mg/kg body weight).
5. *Phaleria macrocarpa* extract+ deferiprone without dose reduction (DPM-1): iron-overloaded rats treated with the combination of ethanol extract of *Phaleria macrocarpa* fruit (100 mg/kg body weight) and deferiprone (462.5 mg/kgBW).
6. *Phaleria macrocarpa* extract+ deferiprone with dose reduction (DPM-2): iron-overloaded rats treated with the combination of ethanol extract of *Phaleria macrocarpa* fruit (100 mg/kg body weight) and deferiprone of half the usual dose (231.25 mg/kg rat weight).

The deferiprone dose for rats (462.5 mg/kg BW) was determined by converting the human dose (75 mg/kg BW) using the human equivalent dose (HED) formula[29]. The PM extract dose (100 mg/kg BW) was selected based on Verna's research, which demonstrated significant reductions in plasma iron levels at this dosage[28].

Plant Extraction

Extraction of *Phaleria macrocarpa* fruit was conducted following the method employed by Verna. The maceration technique was utilized, using 70% ethanol as the solvent to yield an extract with higher flavonoid and phenolic content. A total of 1000.05 grams of dried *Phaleria macrocarpa* fruits were macerated with 70% ethanol at the Balai Penelitian Tanaman Rempah dan Obat (Balittro) Bogor, resulting in an ethanol extract yield of 34.59% (crude extract). Subsequently, concentration was performed at the Laboratory of the Center for Biofarmaka Studies, IPB, using a rotary evaporator for 6 hours, resulting in 81.5833 grams of concentrated ethanol extract (28). The concentrated extract was then diluted with distilled water to achieve a solution with a concentration of 50 mg/mL.

Iron-Overload Induction and Treatment

Iron overload induction for the IO, D, PM, DPM-1, and DPM-2 groups involved intraperitoneal injections of 0.3 mL iron dextran (containing 15 mg Fe) twice a week for three weeks. This method, based on previous research, has been proven to elevate plasma iron levels up to 40 times and ferritin levels up to 10 times higher than the normal group (29,30). After the three-week induction period, the experimental treatments were administered to the respective groups until the 8th week, while iron overload induction persisted until the completion of the study at the 8th week (30).

For the DPM-1 and DPM-2 groups, the test substance was prepared by mixing ethanol extract of *Phaleria macrocarpa* fruit and deferiprone in a microtube, vortexed, and then administered to the experimental animals. The volume of the test substance given to the rats was adjusted to ensure the dosage corresponded to their body weight. All test substances were administered orally once a day using a gastric tube, beginning from the 3rd week until the 8th week of the study.

Hematological Analysis

Blood samples were collected in the 3rd and 8th weeks of the study. Prior to the procedure, rats were anesthetized with ketamine/xylazine. Once anesthesia was achieved, blood was collected from the retro-orbital sinus of the eye using a hematocrit tube inserted and pressed at a 45° angle at the outer corner of the eyes. The blood was then collected up to 1.5 mL in a lithium heparin-containing vacutainer. Subsequently, the blood samples were analyzed using a hematological analyzer (Onetech Med, Model No.A9).

The following hematological parameters were examined: total white blood cell count (WBC) in x10^3/µl, lymphocytes (LYM) in x10^3/µl, granulocytes (GRAN) in x10^3/µl, platelet count (PLT) in x10^3/µl, red blood cell count (RBC) in x10^6/µl, hemoglobin (Hb) in g/dL, mean corpuscular volume (MCV) in femtoliters (fL), mean corpuscular hemoglobin (MCH) in picograms (pg), and mean corpuscular hemoglobin concentration (MCHC) in g/dL.

Statistical Analysis

Data analysis was performed using SPSS version 26. The mean differences among treatment groups (a total of 6 groups) for each parameter at week 3 (baseline) were assessed using one-way ANOVA for normally distributed and homogenous data, Welch's ANOVA for normally distributed but non-homogenous data, and Kruskal-Wallis for non-normally distributed data. On the other hand, the mean differences between week 8 and week 3 for each treatment group (week 8 vs. week 3) were analyzed using dependent t-tests for normally distributed data and Wilcoxon tests for non-normally distributed data. All graphs were generated using the GraphPad Prism program (GraphPad Software, Inc., San Diego, CA).

RESULTS AND DISCUSSION

Peripheral Blood Counts in Iron-Overloaded Mice after 3 Weeks of Induction

To investigate the effect of iron overload on the hematopoietic system, we examined peripheral blood hematological parameters in all iron-overload groups (IO, D, PM, DPM-1, and DPM-2) and the control group. The results are presented in Table 1. Statistical tests indicated that the induction of iron overload for 3 weeks did not cause significant changes in hematological parameters in the control, IO, D, PM, DPM-1, and

DPM-2 groups ($p > 0.05$). Most values were within the normal range or slightly below or above the normal values. One sample from the control group was excluded due to sample lysis and inability to evaluate, resulting in a sample size of only 4 in the control group.

Table 1. Hematological parameters at 3rd week (mean±standard deviation)

Parameter	C	IO	D	PM	DPM-1	DPM-2	p-value
WBC ($x10^3$/µl)	12,85 ± 3,86	12,76 ± 3,19	12,32 ± 2,54	14,98 ± 2,32	12,76 ± 5,07	13,54 ± 4,61	0,892
LYM ($x10^3$/µl)	6,55 ± 1,85	5,78 ± 1,78	6,02 ± 2,47	7,98 ± 1,02	6,80 ± 3,88	6,88 ± 3,94	0,531
GRAN ($x10^3$/µl)	4,20 ± 1,61	4,70 ± 0,90	4,50 ± 0,96	4,78 ± 1,22	3,94 ± 0,70	4,70 ± 0,66	0,765
PLT ($x10^3$/µl)	1167,50± 490,09	925,60± 437,33	868,60± 686,00	1235,40± 86,19	1171,40± 377,75	926,40± 218,42	0,205
RBC ($x10^6$/µl)	8,61 ± 1,23	8,25 ± 1,02	8,25 ± 1,84	9,15 ± 0,35	8,95 ± 0,92	8,63 ± 0,92	0,756
Hb (g/dL)	17,83 ± 2,76	17,40 ± 1,72	18,64 ± 2,50	18,90 ± 0,73	18,98 ± 1,50	18,40 ± 1,42	0,733
MCH (pg)	20,65 ± 0,49	21,14 ± 1,00	23,06 ± 3,09	20,60 ± 0,43	21,22 ± 0,61	21,30 ± 0,77	0,296
MCHC (g/dl)	33,80 ± 1,16	33,66 ± 2,06	34,88 ± 3,43	32,02 ± 0,81	33,22 ± 1,32	33,22 ± 2,43	0,209
MCV (fL)	61,20 ± 1,80	62,88 ± 1,31	65,98 ± 3,62	64,46 ± 1,82	63,92 ± 1,60	64,46 ± 2,51	0,077

Peripheral Blood Counts after Treatment

Various studies indicate that deferiprone, as an iron-chelator, has side effects on the hematologic system. To investigate (1) the effects of deferiprone on the hematologic system in iron-overload conditions, (2) the potential of the ethanol extract of *Phaleria macrocarpa* fruit to improve hematologic profiles in iron-overload conditions, and (3) the potential of ethanol extract of *Phaleria macrocarpa* fruit to reduce the side effects of deferiprone in iron overload conditions, we examined hematological parameters of peripheral blood after 5 weeks of treatment across all treatment groups and compared the changes between weeks 3 and 8 in each group. The results can be seen in Figure 1. One sample from the control group was excluded due to sample lysis and the inability to evaluate, resulting in a sample size of only 4 in the control group.

As expected, rats that were not induced with iron overload and did not receive any treatment in the control group (C) did not show significant changes in hematologic parameters between week 8 and week 3. Meanwhile, the iron-overloaded group without any treatment (IO) exhibited a significant increase in MCV at week 8 compared to week 3. Although not significant, there was a trend of decrease in other hematological parameters, namely WBC, GRAN, PLT, RBC, Hb, MCH, and MCHC, accompanied by an increase in lymphocytes.

The administration of deferiprone alone to iron-overloaded rats resulted in a significant decrease in RBC and Hb at week 8 compared to week 3. In other hematological parameters, although not significant, there was a trend of decrease in WBC, LYM, GRAN, PLT, MCH, and MCHC, and a slight increase in MCV. On the other hand, the administration of ethanol extract of *Phaleria macrocarpa* fruit to iron-overloaded rats led to a significant decrease in PLT, RBC, and Hb with significant increase in MCH and MCV at week 8 compared to week 3. Although not significant, other parameters showed a decreasing trend, including WBC, LYM, and GRAN, while MCHC exhibited a slight increase.

The combination of ethanol extract of *Phaleria macrocarpa* fruit and deferiprone at usual dose resulted in a significant decrease in GRAN, RBC, Hb, and MCHC, as well as a significant increase in MCV at week 8 compared to week 3. Although not significant, other parameters showed a decreasing trend, including WBC, LYM, PLT, and a slight increase in MCH. When the dose of deferiprone was reduced to half of the usual dose, the combination with ethanol extract of *Phaleria macrocarpa* fruit caused a significant decrease in GRAN, PLT, RBC, and Hb, as well as a significant increase in MCV at week 8 compared to week 3. Although not significant, there were slight increase in MCH and slight decrease in WBC, LYM and MCHC.

Several studies suggest that iron overload has a suppressive effect on hematopoiesis. However, in this study, the 3-week injection of iron dextran may not have been sufficient to induce significant changes in peripheral blood counts. Chai's study concludes that damage resulting from iron overload occurs gradually [7]. Okabe et al.'s research showed that in a model injected with iron for 4 weeks, peripheral blood counts were also not remarkably changed. Although platelet levels significantly increased, they remained within normal limits. Meanwhile, WBC, RBC, and Hb did not differ significantly between the IO and control groups in the same study (31).

In this study, following an initial induction period of 3 weeks, we proceeded with the induction process while simultaneously commencing therapy, which extended until the 8th week. For clarity of understanding, we will discuss the effects of therapy administration based on treatment groups.

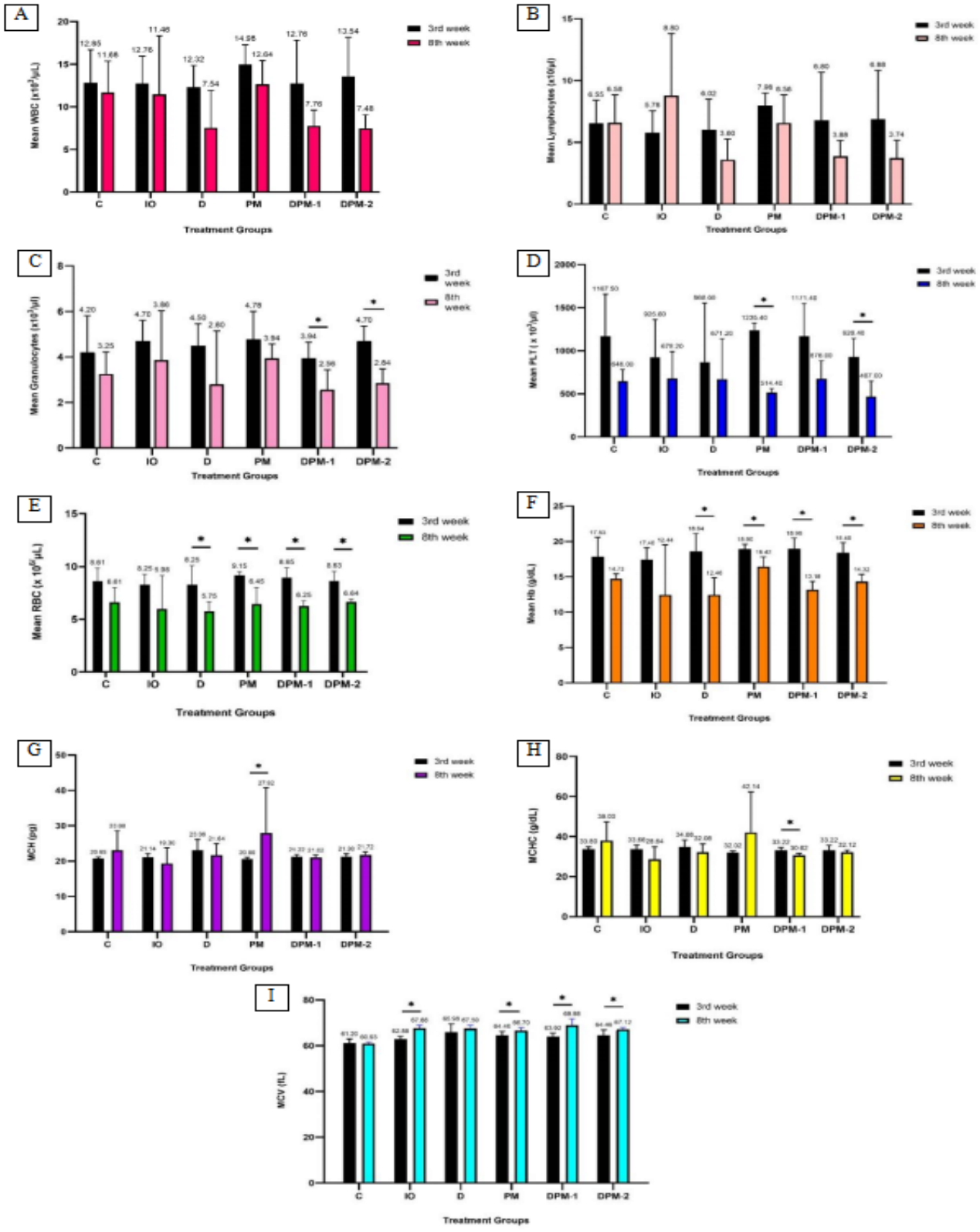
A
Mean WBC (x10³/µL)
12.85
11.66
12.76
11.46
12.32
7.54
14.95
12.64
12.76
7.76
13.54
7.48
3rd week
8th week
C
IO
D
PM
DPM-1
DPM-2
Treatment Groups
B
Mean Lymphocytes (x10/µl)
6.55
6.58
5.78
9.80
6.02
3.60
7.96
6.56
6.80
3.88
6.88
3.74
3rd week
8th week
Treatment Groups
C
Mean Granulocytes (x10³/µl)
4.20
3.25
4.70
3.86
4.50
2.60
4.78
3.94
3.94
2.56
4.70
2.84
3rd week
8th week
Treatment Groups
D
Mean PLT (x 10³/µl)
1167.50
646.00
905.60
679.20
868.00
671.20
1235.40
514.40
1171.40
676.00
928.40
487.60
3rd week
8th week
Treatment Groups
E
Mean RBC (x 10⁶/µL)
8.61
8.61
8.25
8.98
8.25
5.75
9.15
8.45
8.85
8.25
8.83
8.64
3rd week
8th week
Treatment Groups
F
Mean Hb (g/dL)
17.63
14.73
17.48
12.44
18.94
12.46
18.90
16.47
18.99
13.18
18.48
14.32
3rd week
8th week
Treatment Groups
G
MCH (pg)
30.88
31.99
21.14
19.90
23.39
21.64
30.88
37.92
21.33
21.40
21.30
21.72
3rd week
8th week
Treatment Groups
H
MCHC (g/dL)
33.80
38.03
33.86
28.84
34.89
32.08
32.02
42.14
33.22
30.82
33.22
32.12
3rd week
8th week
Treatment Groups
I
MCV (fL)
61.20
60.93
62.98
67.66
65.99
67.50
64.48
66.52
63.90
68.86
64.48
67.12
3rd week
8th week
Treatment Groups

Figure 1. Changes in hematological parameters from week 3 to week 8 in all treatment groups (*p<0.05)

Iron-overloaded group without treatment (IO). The significant increase in MCV in iron-overloaded rats in this study is supported by Sadek et al.'s research, which also showed a significant increase in MCV in mice induced with ferric hydroxide polymaltose complex for 4 weeks. An elevated MCV indicates an increase in red blood cell size. However, in their study, the increase was not limited to MCV but also observed in RBC, Hb, HCT, and MCH, indicating a possible compensatory mechanism to safely sequester excess iron, such as increasing the size and concentration of hemoglobin within red blood cells (MCH) (32,33). However, in this study, other hematologic parameters experienced a decrease, suggesting a potential damage to hematopoietic progenitor cells that leads to the decrease in those parameters (except for MCV and lymphocytes).

In Zhou et al.'s study, iron-overload induced by iron-dextran for 4 weeks in mice led to a decrease in Hb, platelet count, and WBC. Iron-overload conditions resulted in reduced viability and proliferation activity of bone marrow mononuclear cells, mediated by the downregulation of SIRT3 expression and activity. SIRT3 regulates SOD2 (an antioxidant found exclusively in mitochondria) activity by modulating the acetylation level of SOD2. Reduced SIRT3 expression and activity led to increased acetylation of SOD2, reducing its activity and causing an increase in free radicals in mitochondria. This, in turn, led to mROS-dependent autophagic cell death and bone marrow damage (8).

Taoka et al.'s investigation demonstrated that in situations of iron overload, the development of erythroid burst-forming unit colonies and the maturation of erythroblasts were significantly inhibited. These effects were alleviated by iron chelation with deferoxamine (DFO). Furthermore, excessive iron burden induced apoptosis in immature erythroblasts by increasing intracellular reactive oxygen species (ROS) levels (34). Additionally, the high ratio of polyunsaturated fatty acids to total lipids in erythrocytes and erythrocyte membranes suggests susceptibility to lipid peroxidation. Moreover, red blood cells are particularly prone to lipid peroxidation due to their continuous exposure to high oxygen tension and the presence of elevated iron ion concentrations (35).

Despite the lack of remarkable influence on peripheral blood counts, the observed decreasing trend in this study could also be attributed to changes in the microenvironment due to iron overload. Okabe et al.'s research demonstrated that iron overload conditions disturb the hematopoietic microenvironment, marked by a decrease in mRNA levels of various cytokines, chemokines, and adhesion molecules involved in hematopoiesis, such as CXCL12, VCAM-1, IGF-1, and stem cell factor (SCF), by up to 1/20 compared to normal controls. Additionally, there was a reduction in erythropoietin protein levels and thrombopoietin mRNA. Thrombopoietin, produced in the liver, plays a role in platelet production. The decrease in thrombopoietin levels may be caused by iron accumulation in the liver, leading to impaired thrombopoietin production (31).

Meanwhile, in this study, there was an increase in lymphocytes. This finding is supported by Chai et al.'s research, which demonstrated an increase in the percentage of lymphocytes in iron-overloaded mice compared to the normal mouse group[7]. A study by de Souza Aquino et al. also showed an increase in lymphocytes in rats induced with FeSO4 administration. The rise in lymphocytes likely occurs in response to the injury caused by excess FeSO4 administered to the rats, as lymphocytes serve as a primary defense mechanism against the harmful effects of excess iron (36). This is mediated by the ability of lymphocytes to uptake non-transferrin-bound iron (NTBI). Furthermore, the iron is likely stored in ferritin, consistent with evidence demonstrating that T lymphocytes can synthesize H-ferritin (37).

Deferiprone-treated group (D). Changes in hematological parameters in this group may occur as a result of both iron-overload conditions and the side effects of deferiprone. As previously explained, iron-overload conditions can lead to damage to hematopoietic progenitor cells, both white blood cells and red blood cells, and disrupt proteins necessary for platelet production, resulting in a decrease in related parameters (8,31,34). Meanwhile, the increase in mean corpuscular volume (MCV) could represent a compensatory mechanism aimed at safely sequestering excess iron (32,33).

In addition to iron-overload conditions, changes in hematological parameters in this group may be attributed to the side effects of deferiprone. Hu et al.'s study demonstrated that deferiprone is toxic to hematopoietic stem and progenitor cells (HPSC) (15). Another study by Vlachaki et al. indicated that the addition of serum from patients treated with deferiprone to progenitor cell cultures of the granulocytic lineage resulted in maturation arrest in those cell cultures. The research showed a decrease in the number of progenitor cell colonies of the granulocytic lineage (38). Therefore, there is a possibility that damage to progenitor cells caused by deferiprone may contribute to the decline in hematological parameters in the deferiprone-treated group.

***Phaleria macrocarpa*-treated group (PM).** The administration of ethanol extract of *Phaleria macrocarpa* fruit to iron-overloaded rats resulted in a significant decrease in PLT by 58,36%. The decrease in platelet count observed in this group is the most significant compared to other groups, including the untreated iron-overloaded (IO) group. This decline in platelet count may be attributed to the induction of iron-overload and the side effects of the extract on platelets. It is possible that there are other compounds within the extract that synergistically contribute to the reduction in platelet count. Based on research by Hendra et al., *Phaleria macrocarpa* fruit contains naringin

(20). A study by Li et al. regarding the toxicity of naringin showed that although not significant, there was a trend of decreased platelet count in male beagle dogs administered naringenin for 3 and 6 months (39). Additionally, the ethanol extract of *Phaleria macrocarpa* fruit also contains naringenin (21), which has been demonstrated to decrease platelet counts in diabetic rats (40).

Meanwhile, compared to other groups, only this group demonstrated concurrent increases in mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV). Additionally, the decrease in hemoglobin (Hb) in this group was also the smallest compared to other groups. As previously discussed, the simultaneous increase in MCH, MCV, and MCHC under conditions of iron-overload suggests the possibility of compensatory mechanism to safely sequester excess iron, one of which is by increasing the size and concentration of hemoglobin in red blood cells (32,33). Therefore, it is plausible that the active compounds present in the ethanol extract of *Phaleria macrocarpa* fruit may aid in optimizing this compensatory mechanism.

When compared to the untreated iron-overloaded (IO) group, the decreases in WBC, GRAN, RBC do not differ significantly. Thus, it can be assumed that the reduction in these parameters in this group is likely attributable to the condition of iron overload rather than the side effects of the administration of the ethanol extract of *Phaleria macrocarpa* fruit. The extract of *Phaleria macrocarpa* fruit is known to have a protective effects on both white blood cells and erythrocytes. Research conducted by Muruganandan and Gupta has underscored the protective properties of mangiferin against cyclophosphamide-induced leucopenia and erythrocytopenia in rats (19). Rodriguez's investigation demonstrated that mangiferin enhanced erythrocyte resistance to H2O2-induced reactive oxygen species production (35). The research conducted by Hazalin et al. indicates that the extract of *Phaleria macrocarpa* fruit possesses high antioxidant capacity and is capable of providing protective effects against oxidative stress (41).

Specifically, regarding lymphocytes (LYM), a decrease was observed in this group, whereas a significant increase was noted in the untreated iron-overloaded (IO) group. Intriguingly, mangiferin compounds present in the extract are known to enhance the survival of lymphocytes exposed to H2O2 (19). Therefore, the decline in lymphocytes in this context might be attributed to other constituents within the extract. The ethanol extract of *Phaleria macrocarpa* fruit contains epigallocatechin-gallate (EGCG), which is recognized for its suppressive effects on lymphocytes. Findings from Munkyong et al.'s study indicated that compared to mice fed a control diet, those fed with 0.3% EGCG exhibited reduced lymphocyte proliferation and inhibited T cell division and cell cycle progression (42).

When compared to deferiprone, the decrease in white blood cell count (WBC), lymphocytes (LYM), and granulocytes (GRAN) in this group was considerably smaller. Therefore, it can be inferred that the adverse effects of the ethanol extract of *Phaleria macrocarpa* fruit on WBC, LYM, and GRAN are not as pronounced as those of deferiprone.

***Phaleria macrocarpa*+Deferiprone-treated group (DPM-1 and DPM-2).** Based on the results, the magnitude of parameter changes in the DPM-2 group appears smaller compared to the DPM-1 group, except for white blood cell count (WBC) and platelet count (PLT). For instance, the decrease in granulocytes in the DPM-1 group was 46.44%, whereas in the DPM-2 group, it was 39.57%. This difference may be attributed to the fact that in the DPM-2 group, the dose of deferiprone was reduced by half from the standard dosage. As previously explained, deferiprone is recognized for its toxicity to hematopoietic stem and progenitor cells (HPSC) (15) and has been shown to decrease the number of progenitor cell colonies of the granulocytic lineage (38).

Additionally, a noteworthy discovery in this study is that the combination of deferiprone with the ethanol extract of *Phaleria macrocarpa* fruit (DPM-1 and DPM-2) resulted in a significant reduction in granulocytes, whereas when administered separately, the decrease in both parameters was not significant. When examined individually, the administration of deferiprone alone led to a decrease in granulocytes by 37.78%, while the administration of the ethanol extract of *Phaleria macrocarpa* fruit resulted in a decrease of 17.57%. The decrease in granulocytes in the PM group was equivalent to that in the IO group. This suggests that the reduction in granulocytes in the DPM-1 and DPM-2 groups may be more attributable to the condition of iron overload and the side effects of deferiprone, and the addition of the extract may not have been sufficient to ameliorate this condition. This notion is further supported by the data from the DPM-2 group, where a reduction in the dose of deferiprone led to a smaller decrease in granulocytes compared to the DPM-1 group

CONCLUSION

As a conclusion, this study highlights the hematologic modulatory properties of the ethanol extract derived from *Phaleria macrocarpa* fruit. Both deferiprone and the extract were found to induce alterations in various hematologic parameters when administered independently. Additionally, combining deferiprone with the extract led to further modifications, potentially indicative of dose-dependent effects from deferiprone. Further research is needed to elucidate the mechanism of action of the combination of *Phaleria macrocarpa* fruit extract and deferiprone on the hematologic system, thereby allowing for more conclusive conclusions to be drawn, including the possibility of interactions.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose**.**

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